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Virology features of a family cluster of SARS-CoV-2 infections in Shanghai, China

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ABSTRACT

The global spread of SARS-CoV-2 is currently continuing, and the World Health Organization has announced the risk assessment of the viruses as high. In this study, we analyzed virology features of SARS-CoV-2 causing a family cluster outbreak. Among the six family members, five have been laboratory-confirmed infection of SARS-CoV-2 viruses. A total of five SARS-CoV-2 viruses have been isolated from the nasopharyngeal swabs. The complete genome of the viruses exhibited 100% nucleotide identity with each other. Only two nucleotide differences have been observed between genomes of the isolated viruses and the HCoV/Wuhan/IVDC-HB-01/2019 strain. Therefore, SARS-CoV-2 has been confirmed as the causation of the family cluster infections. © 2021 Chinese Medical Association Publishing House. Published by Elsevier BV. This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>).

1. Introduction

The coronavirus epidemic outbreak has currently caused great worldwide concern. Coronaviruses are single-strand, positive-sense RNA genomes ranging from 26 to 32 kilobases in length [1]. Coronaviruses can infect several birds and various mammals, including camels, bats, masked palm civets, mice, dogs, and cats [2,3]. Coronavirus could cause different clinical diseases involving respiratory, enteric, hepatic, and neurological systems [4]. When infected, people exhibited mild clinical symptoms [1]. However, the severe acute respiratory syndrome coronavirus (SARS-CoV) and the Middle East respiratory syndrome coronavirus (MERS-CoV) have caused more than 8,000 human infections (916 fatalities) and 2,574 human cases (886 deaths) by the end of April 2021 respectively. (from WHO resource, accessed May 16, 2021 [5]).

At the beginning of 2020, a new coronavirus was identified [6–8]. The virus has about 75%–80% homology to SARS-CoV, but divergent from MERS-CoV, wherefore it was named as SARS-CoV-2 [9,10]. As of May 16, 2021, China has reported 90,872 laboratory-confirmed cases of SARS-CoV-2 infection across 31 provinces and Xinjiang Production and Construction Corps, with 4,636 fatalities (The information is from the official website of the National Health Council, accessed May 16, 2021). In addition, 223 countries and regions throughout

the world have reported a total of 162,177,376 SARS-CoV-2 human cases, with a fatality rate of 2.07% (from WHO Coronavirus Dashboard, accessed May 16, 2021 [11]).

On January 19, 2020, the first SARS-CoV-2 case was reported in Shanghai. Up to May 16, 2021, a total of 2,038 cases with seven deaths have been documented in Shanghai (The information is from the official website of the Shanghai Municipal Health Commission, accessed May 16, 2021). This study reported epidemiologic features and virological characteristics of a family cluster of SARS-CoV-2 infections in Shanghai, China.

2. Case presentation

As field investigators and clinicians, we used a standardized surveillance reporting form to gather epidemiological and clinical data. Nasopharyngeal swabs and fecal samples were collected for viral isolation and other tests based on the Technical Guidelines for Laboratory Testing of Novel Coronavirus Pneumonia.

The cluster in our study involved five confirmed COVID-19 cases and a timeline to illustrate the details (Fig. 1). The first patient (case A) was back in Shanghai from Wuhan on January 17. Two days later, he developed physical discomfort and fatigue but did not go to the hospital. His body temperature was average and with no pulmonary symptoms. On January 27, three other families (case B, C, D) members also developed fever and were laboratory confirmed as SARS-CoV-2 infection. On the following day, case E was home quarantine, together with a 3-year-old girl (the daughter of case E), till her confirmation to be infected with SARS-CoV-2 on the 18th day after the first patient's

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returning to Shanghai. However, the 3-year-old girl was negative for the SARS-CoV-2. Detailed epidemiological and clinical characteristics of the cases are described in Table 1.

The Ion Torrent S5 platform (ThermoFisher, Waltham, USA) was used for whole-genome sequencing with a mean read length of about 200 bp and at least 10,000 depth of sequence. Data analysis was predominantly conducted using CLC Genomics Workbench 7.5.1 software. Low-quality reads were trimmed using CLC trimmer, with a quality limit set at 0.05. The filtered reads were de novo-assembled in CLC under default parameters. Contigs with coverage over 10 were extracted and blasted against the Global Initiative on Sharing All Influenza Data (GISAID) databases. The phylogenetic and genetic characteristics of the SARS-CoV-2 genome sequences from the family cluster were analyzed. The alignment showed that viral nucleotide sequences of this cluster shared 100% sequence similarities. The genome of the five viral strains showed 99.99% nucleotide identities compared with that of SARS-CoV-2. Wuhan-Hu-1 reference strain (GenBank mn908947), and only two nucleotides were different, at site 8,782 and 28,144 of ORF1ab and ORF8, respectively.

According to this molecular typing method, the five strains in our study were divided into S-lineage (nt8782T and nt28144C), which was the main epidemic type in the early stages of the outbreak. It is speculated that S-lineage might be an older type.

3. Discussion and conclusion

Although many studies have been performed to characterize the SARS-CoV-2 virus, including its basic biology, treatments, animal source and vaccines, and so on [12–15], there are still many unknowns to be revealed. Wearing a mask and social distancing could break the transmission [16–19]. This study analyzed the epidemiologic and virologic features of SARS-CoV-2 viruses isolated from a family cluster. Five in six members have been infected with SARS-CoV-2, except one 3-year-old girl. Neither virus nucleotide nor antibody has been detected from the girl. Due to the limit of evidence, we could not confirm why the girl was not infected. However, during the close contact tracing of these SARS-CoV-2 cases, personal protections, including masks and gloves, and the disinfection measures were well carried out for the girl to keep her away from the viral infections.

Patients in our study had an incubation period of approximately 7–18 days. Case B, who was 55 years old, exhibited severe infections

during hospitalization. The remaining four patients showed mild symptoms after infection, indicating that age might be an essential factor associated with the clinical outcome of SARS-CoV-2 conditions. Of note, fecal samples of case C have been continuously positive for SARS-CoV-2 viruses for 26 days. Although no SARS-CoV-2 virus has been isolated from any fecal samples, the long-term virus shedding via the intestine verifies the implementation of effective public health measures for disease prevention and control.

In summary, our results highlighted the contribution of personal protection equipment in disease prevention and control. In the epidemiological investigation, the evidence provided by case reports was less persuasive than results of well-designed studies wherein information was obtained following a specified protocol. The whole-genome sequence evidence provided the basis for the reliability of the transmission of the cluster in our study.

In addition, more attention should be paid to the fecal-oral transmission of SARS-CoV-2. Currently, limited knowledge is known for the SARS-CoV-2. More studies are urgently needed to prevent future epidemics of the virus.

Ethics statement

The experiments and protocols in this study were screened and approved by the Ethical Review Committee of the Shanghai Municipal Centre for Disease Control and Prevention (Shanghai CDC, ethical number: 2020-39). Clinical specimens were obtained from the routine surveillance programme of the Shanghai CDC. Sample collection was approved by either the patients or their parents with prior informed consent.

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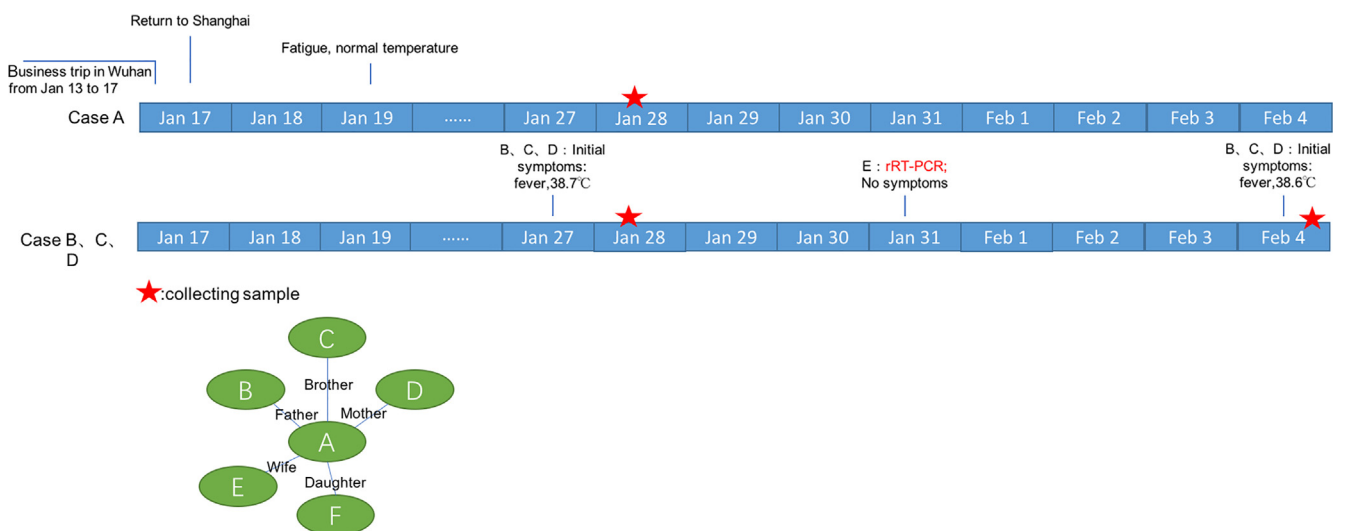


Fig. 1. Schematic diagram of family dissemination and timeline of exposure to pre-symptomatic case and illness onsets of cases in Cluster of COVID-19 outbreak, Shanghai, China, 17 January – 4 February 2020 (n = 5).

Table 1
Detailed epidemiological and clinical characteristics of the cases and laboratory test results.

Category	Case A	Case B	Case C	Case D	Case E	Case F
Relationship	Son of case B and D	Father of case A and C	Daughter of case B and D	Mother of case A and C	Wife of case A	Daughter of case A
Age (years)	28	55	27	53	27	3
Sex	Male	Male	Female	Female	Female	Female
Contact history with Wuhan	Yes (business trip in Wuhan from Jan. 13 to 17)	No	No	No	No	No
Date of onset	Jan. 19–20 (fatigue)	Jan. 27	Jan. 27	Jan. 27	Feb. 4	Asymptomatic
Symptom	Normal temperature and CT	Fever	Fever	Fever	Fever	The antibody test was negative
Date of admission	Jan. 28	Jan. 28	Jan. 28	Jan. 28	Feb. 4	
Presenting symptoms and signs						
Fever	Yes	Yes	Yes	Yes	Yes	
Body temperature (°C)	37.5	38.7	38.2	38.5	38.6	
Cough	Yes (dry)	No	Yes (dry)	Yes (dry)	Yes (dry)	
Coryza	No	Yes (mild)	No	No	No	
Muscle soreness	Yes	Yes	No	Yes	No	
Imaging features of pneumonia yes	Yes	Yes	Yes	Yes	Yes	
White blood cell count ($\times 10^9$ cells per L); (normal range 3.9–9.9)	4.49×10^9	8.80×10^9	3.88×10^9	5.79×10^9	5.51×10^9	
lymphocyte count ($\times 10^9$ cells per L); (normal range 1.1–3.6)	0.91×10^9	0.78×10^9	0.94×10^9	2.71×10^9	0.85×10^9	
Severe	No	Yes	No	No	No	
Date of discharge	Feb. 7	Feb. 4	Mar. 3	Feb. 24	Feb. 22	
Virological testing						
Sample type	Throat swab	Throat swab	Throat swab	Throat swab	Throat swab	
Collection date	Jan. 28	Jan. 28	Jan. 28	Jan. 28	Feb. 4	
Virus isolation	Yes	Yes	Yes	Yes	Yes	
Sequence obtained	Complete	Complete	Complete	Complete	Complete	

Conflict of interest statement

The authors declare that there are no conflicts of interest.

Author contribution

Yanqiu Zhou: Conceptualization, Data Curation, Investigation, Writing - Original Draft, Writing - Review & Editing. **Zheng Teng:** Conceptualization, Data Curation, Investigation, Writing - Original Draft, Writing - Review & Editing. **Hongyou Chen:** Conceptualization, Data Curation, Investigation, Writing - Original Draft, Writing - Review & Editing. **Xiaoxian Cui:** Data Curation, Formal Analysis. **Fanghao Fang:** Investigation, Data Curation. **Jiabin Mou:** Investigation, Data Curation. **Hui Jiang:** Investigation, Data Curation. **Xi Zhang:** Conceptualization, Funding Acquisition.

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